Docket No. 3203.17330-PCT-US

CHAPTER II

09/743930

JC06 Rec'd PCT/PTO 17 JAN 2001

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US) (ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II)

PCT/CA99/00651	16 July 1999	17 July 1998
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED

Polyionic Hydrogels Containing Xanthane and Chitosan
TITLE OF INVENTION

Esteban Chornet and Severian Dumitriu
APPLICANT(S)

Box PCT
Assistant Commissioner for Patents
Washington D.C. 20231

ATTENTION: EO/US

NOTE: *The completion of those filing requirements that can be made at a time later than 30 months from the priority date results from the Commissioner exercising his judgment under the authority granted under 35 USC 371(o. 7-he riling receipt will show the actual date of receipt of the last item completing the entry into the national phase. See 37 CFR 1.491 which states: 'An international application enters the national state when the applicant has riled the documents and fees required by 35 USC 371(c) within the periods set forth in § 1.494 and § 1.495.'*

WARNING: *Where the items are those which can be submitted to complete the entry of the international application into the national phase are subsequent to 30 months from the priority date the application is still considered to be in the international state and if mailing procedures are utilized to obtain a date the express mail procedure of 37 CFR 1.10 must be used (since international application papers are not covered by an ordinary certificate of mailing - See 37 CFR 1.8.*

NOTE: *Documents and fees must be clearly identified as a submission to enter the national state under 35 USC 371 otherwise the submission will be considered as being made under 35 USC 111. 37 CFR 1.494(f).*

CERTIFICATION UNDER 37 C.F.R. 1.10

I hereby certify that this Transmittal Letter and the papers indicated as being transmitted therewith is being deposited with the United States Postal Service on this date 17 January 2001, in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EL 746527605 US, addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Julie A. Wolf
(Type or print name of person mailing paper)

Julie A. Wolf
(Signature of person mailing paper)

WARNING. *Certificate of mailing (first class) or facsimile transmission procedures of 37 C.F.R. 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.*

WARNING: *Each paper or fee filed by 'Express Mail' must have the number of the 'Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. 1.10(b).*
'Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will not be granted on petition. " Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.

1. Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. 371:

   a. [x] This express request to immediately begin national examination procedures (35 U.S.C. 371(f)).

   b. [x] The U.S. National Fee (35 U.S.C. 371(c)(1)) and other fees (37 CFR 1.492) as indicated below:

2. Fees

CLAIMS FEE	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCU-LATIONS
	TOTAL CLAIMS	16 -20	0	X 18.00	$0.00
	INDEPENDENT CLAIMS	1 -3	0	X78.00	$0.00
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			$260.00	$0.00
BASIC FEE	[ ] US PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where an International preliminary examination fee as set forth in §1.482 has been paid on the international application to the US PTO: [ ] and the international preliminary examination reports states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 CFR 1.492(a)(4)). **$96.00** [ ] and the above requirements are not met (37 CFR 1.492(a)(1)) **$670.00** [X] US PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY Where no international preliminary examination fee as set forth in §1.482 has been paid to the US PTO, and payment of an international search fee as set forth in §1.445(a)(2) to the US PTO: [ ] has been paid (37 CFR 1.492(a)(2)) **$760.00** [ ] has not been paid (37 CFR 1.492(a)(3)) **$970.00** [X] where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 CFR 1.492(a)(5)) **$840.00**				$840.00
				Total of above Calculations	$840.00
SMALL ENTITY	Reduction by ½ for filing by small entity, if applicable. Affidavit must be filed also (Note 37 CFR 1.9, 1.27, 1.28)				-$420.00
				Subtotal	$420.00
				Total National Fee	$420.00
	Fee for recording the enclosed assignment document (37 CFR 1.21(h)). See Item 13 below). See attached "ASSIGNMENT COVER SHEET". **$40.00**				
TOTAL				TOTAL FEES ENCLOSED	$615.00

i. [x] A check in the amount of 615.00 to cover the above fees is enclosed. (Includes surcharge for late filing of Declaration / Power of Attorney and Petition fee for submitting non-English specification.)

ii. [ ] Please charge Account No._____ in the amount of $_____ A duplicate copy of this sheet is enclosed.

*WARNING: To avoid abandonment of the application the applicant shall furnish to the United States Patent and Trademark Office not later than the expiration of 30 months from the priority date: * * * (2) the basic national fee (see §1.492(a)). The 30-month time limit may not be extended." 37 CFR §1.495(b).*

WARNING: If the translation of the international application andlor the oath or declaration have not been submitted by the applicant within thirty (30) months from the priority date, such requirements may be met within a time period set by the Office. 37 CFR § 1.495(b)(2). The payment of the surcharge set forth in § 1.492(e) is required as a condition for accepting the oath or declaration later than thirty (30) months after the priority date. The payment of the processing fee set forth in § 1.492(o is required for acceptance of an English translation later than thirty (30) months after the priority date. Failure to comply with these requirements will result in abandonment of the application. The provisions of § 1. 136 apply to the period which is set. Notice of Jan. 3, 1993, 1147 0.G. 29 to 40.

3. [x] A copy of the International application as filed (35 U.S.C. 371(c)(2)):

NOTE: Section 1.495 (b) was amended to require that the basic national fee and a copy of the international application must be filed with the Office by 30 months from the priority date to avoid abandonment. 'The International Bureau normally provides the copy of the international application to the Office in accordance with PCT Article 20. At the same time, the International Bureau notifies applicant of the communication to the Office. In accordance with PCT Rule 47. 1, that notice shall be accepted by all designated offices as conclusive evidence that the communication has duly taken place. Thus, if the applicant desires to enter the national stage, the applicant normally need only check to be sure the notice from the International Bureau has been received and then pay the basic national fee by 30 months from the priority date.' Notice of Jan. 7, 1993, 1147 O.G. 29 to 40, at 35-36. See item 14c below.

a. [X] is transmitted herewith.

b. [ ] is not required, as the application was filed with the United States Receiving Office.

c. [ ] has been transmitted

i. [ ] by the International Bureau.
Date of mailing of the application (from form PCT/IB/308):_____

ii. [ ] by applicant on_____
Date

4. [X] A translation of the International application into the English language (35 U.S.C. 371(c)(2)):

a. [ ] is transmitted herewith.

b. [ ] is not required as the application was filed in English.

c. [ ] was previously transmitted by applicant on_____
Date

d. [X] will follow.

5. [X] Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371 (c)(3)):

NOTE *The Notice of January 7, 1993 points out that 37 CFR § 1.495(a) was amended to clarify the existing and continuing practice that PCT Article 19 amendments must be submitted by 30 months from the priority date and this deadline may not be extended. The Notice further advises that., 'The failure to do so will not result in loss of the subject matter of the PCT Article 19 amendments. Applicant may submit that subject matter in a preliminary amendment filed under section 1. 121. In many cases, filing an amendment under section 1.121 is preferable since grammatical or idiomatic errors may be corrected.' 1147 O.G. 29-40, at 36.*

a. [ ] are transmitted herewith.

b. [ ] have been transmitted

i. [ ] by the International Bureau.
Date of mailing of the amendment (from form PCT/1 B/308):_____

ii. [ ] by applicant on_____

c. [X] have not been transmitted as

i [X] applicant chose not to make amendments under PCT Article 19. Date of mailing of Search Report (from form PCT/ISA/210.): 05/NOV/99

ii. [ ] the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

6. [X] A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. 371(c)(3)):

a. [ ] is transmitted herewith.

b. [ ] is not required as the amendments were made in the English language.

c. [X] has not been transmitted for reasons indicated at point 5(c) above.

7. [X] A copy of the international examination report (PCT/IPEA/409)

[X] is transmitted herewith.

[ ] is not required as the application was filed with the United States Receiving Office.

8. [X] Annex(es) to the international preliminary examination report

a. [X] is/are transmitted herewith.(if they exist)

b. [ ] is/are not required as the application was filed with the United States Receiving Office.

9. [ ] A translation of the annexes to the international preliminary examination report

a. [ ] is transmitted herewith.

b. [ ] is not required as the annexes are in the English language.

10. [X] An oath or declaration of the inventor (35 U.S.C. 371(c)(4)) complying with 35 U.S.C. 115

a. [ ] was previously submitted by applicant on_____
Date

b. [ ] is submitted herewith, and such oath or declaration

i. [ ] is attached to the application.

ii. [ ] identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3(b) or 3(c) and 5(b); and states that they were reviewed by the inventor as required by 37 C.F.R. 1.70.

iii. [X] will follow.

OTHER DOCUMENT(S) OR INFORMATION INCLUDED:

11. [X] An International Search Report (PCT/ISA/21 0) or Declaration under PCT Article 17(2)(a):

a. [X] is transmitted herewith.

b. [ ] has been transmitted by the International Bureau. Date of mailing (from form PCT/IB/308):

c. [ ] is not required, as the application was searched by the United States International Searching Authority.

d. [ ] will be transmitted promptly upon request.

e. [ ] has been submitted by applicant on_____
Date

12. [X] An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98:

a. [ ] is transmitted herewith.
Also transmitted herewith is/are:
[ ] Form PTO-1449 (PTO/SB/08A and 08B).
[ ] Copies of citations listed.

b. [X] will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. 371(c).

c. [ ] was previously submitted by applicant on_____
Date

13. [ ] An assignment document is transmitted herewith for recording.
A separate"COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" is also attached.

14. [X] Additional documents:

a. [X] Copy of request (PCT/RO/101)

b. [X] International Publication No. WO 00/04086

i. [X] Specification, claims and drawing (translation to follow)

ii. [ ] Front page only

c. [ ] Preliminary amendment (37 C.F.R. § 1.121)

d. [X] Other
Copy of the International Application including modifications
(translation to follow)

15. [X] The above checked items are being transmitted

a. [X] before 30 months from any claimed priority date.

b. [ ] after 30 months.

16. Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on __ namely:

AUTHORIZATION TO CHARGE ADDITIONAL FEES

WARNING: Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges if extra claims are authorized.

[X] The Commissioner is hereby authorized to charge the following additional fees that may be required by this paper and during the entire pendency of this application to Account No. 06-2360.

[X] 37 C.F.R. 1.492(a)(1), (2), (3), and (4) (filing fees)

WARNING: Because failure to pay the national fee within 30 months without extension (37 CFR § 1.495(b)(2)) results in abandonment of the application, it would be best to always check the above box.

[X] 37 C.F.R. 1.492(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.492(d)), it might be best not to authorize the PTO to charge additional claim fees, except possible when dealing with amendments after final action.

[X] 37 C.F.R. 1.17 (application processing fees)

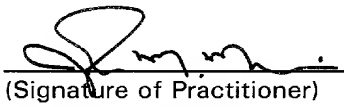
WARNING: While 37 CFR 1. 17(a), (b), (c) and (d) deal with extensions of time under § 1. 136(a), this authorization should be made only with the knowledge that: "Submission of the appropriate extension fee under 37 CFR 1.136(a) is to no avail unless a request or petition for extension is filed." Notice of Nov. 5, 1985 (1060 O.G. 27).

[ ] 37 C.F.R. 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 C.F.R. 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b).

NOTE: 37 C.F.R. 1.28(b) requires 'Notification of any change in loss of entitlement to small entity status must be filed in the application . . . prior to paying, or at the time of paying . . . issue fee.' From the wording of 37 CFR 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

[X] 37 CFR 1.492(e) and (Q (surcharge fees for filing the declaration and/or filing an English translation of an International Application later than 30 months after the priority date).



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09/743930

TITLE OF THE INVENTION

XANTHANE AND CHITOSANE BASED POLYIONIC HYDROGELS FOR THE STABILIZATION AND CONTROLLED RELEASE OF VITAMINS

FIELD OF THE INVENTION

The present invention relates to xanthane and chitosane based hydrogels. More particularly, the present invention relates to dietary complements and to drug delivery devices where such gels are loaded with active agents such as vitamins, nucleic acids, amino acids and oligopeptides.

BACKGROUND OF THE INVENTION

The formation of chitosane or xanthane based hydrogels is known. The use of such hydrogels as an inert support for the immobilization of enzymes, or for the controlled release of certain antibiotics or anti-cancer agents is described in US patents 5,620,706 and 5,648,252. However, the application of xanthane or chitosane based hydrogels in the loading, stabilization and releasing of vitamins, nucleic acids, amino acids and oligopeptides remains to date unexplored.

The conservation of active ingredients susceptible to degradation such as vitamins, nucleic acids, amino acids and oligopeptides, constitutes one of the currant difficulties in the production of dietary complements as well as in dermatology. Exposure to light and heat accelerates this degradation.

Considering the important beneficial potential of these active ingredients, and in order to offer some protection to these active ingredients from degradation or to render them hydrophobic, several vehicles such as tablets, capsules, gel capsules, cremes, ointments, gels, aqueous dispersions (emulsions) and solutions were developed. However, several disadvantages of these synthetic preparations, such as the potential for irritation and toxicity, were alluded to.

Unable to find an adequate vehicle among the conventional excipients, the use of synthetic polycations was recently proposed. For example in European patent application 504 066 A1, 1992, filed by Oreal, France, cosmetic compositions containing a dispersion of solid active particles whose surface is covered with a cationic polymer, are suggested. However, the object of the cationic surface layer is to render the composition as a whole more stable as opposed to stabilizing a particular ingredient facing a tendency towards degradation.

There thus remains an important need to develop a new vehicle useful in food preparations, cosmetology and dermatology holding active photosensitive and thermosensitive active ingredients such as vitamins, nucleic acids, amino acids and oligopeptides. One of the objects of the present invention is to provide dietary and dermatological preparations containing a natural polyionic hydrogel allowing for the protection of photo and thermo-sensitive active ingredients, without exhibiting a potential for irritation or toxicity. Yet another object of the present invention is to provide a method allowing for the incorporation of these active ingredients into a natural polyionic hydrogel.

Other objects, advantages and features of the present invention will become more apparent upon reading the following non-restrictive description of preferred embodiments, which are exemplary and should not be interpreted as limiting the scope of the present invention. It should be understood however, that this detailed description, while indicating preferred embodiments of the invention, is given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art.

SUMMARY OF THE INVENTION

In general terms, the invention provides a thermo and photo stable composition comprised of a hydrogel composed of a xanthane and chitosane complex, wherein the hydrogel comprises at least one thermo or

photo sensitive substance selected from the group consisting of: vitamins, amino acids, nucleic acids and oligopeptides, and wherein the hydrogel is adapted to release the above mentioned thermo- or photo sensitive substances in an animal or human subject. Preferably, the thermo or photo sensitive substances are present in a proportion of 5 to 25% by weight of the total composition. More preferably, these substances are selected from vitamins A, B, C, D, E and K.

The invention also provides a process for the preparation of the inventive compositions, the process comprising the following steps:

(a) dissolving liposoluble substances in an appropriate solvent;

(b) adding this solution, while stirring, to a xanthate solution;

(c) pulverizing the mixture from step (b) to a chitosane solution;

(d) recuperating the so-formed hydrogel;

(e) incorporating by diffusion of the liposoluble substances from step (a) to the hydrogel;

all the steps are carried out essentially in the absence of oxygen and light.

In an other process, according to the present invention, the composition of the invention could be obtained by the following steps:

(a) pulverizing a xanthane solution in a chitosane solution;

(b) recovering the so-formed hydrogel will be characterized by lyophilizing the hydrogel;

(c) introducing the lyophilized hydrogel to an aqueous solution comprising the hydrosoluble substances that can be advantageously stabilized by the addition of amino acids such as L-cysteine, L-cystine and L-methionine or a mixture of the latter or by the addition of tri-peptides;

(d) incorporating the hydrosoluble substances in the hydrogel by diffusion of the hydrosoluble substances during the swelling of the hydrogel, the said hydrogel having a degree of swelling of 2000% or more.

In accordance with the present invention there is also provided a method of use for these hydrogels in dermatology or as a dietary complement.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 Complexation between the chitosane and xanthane

Figure 2 Assembly scheme for the kinetic study

Figure 3 Amount of Vitamin C liberated in function of time
Coded sample VS2L. Hydrogel CHITOSAN^{MC}-Vit. C prepared from CHITOSAN^{MC} with swelling degree (α) = 1800%

Figure 4 Rate of release of vitamin C in function of time.
Coded sample VS2L

Figure 5 Weight of vitamin C in fonction of time
Coded sample VS2R. Hydrogel CHITOSAN^{MC}-Vit. C prepared from CHITOSAN^{MC} with swelling degree (α) = 2200%

Figure 6 Rate of release of vitamin C in function of time.
Coded sample VS2R

Figure 7 Variation of the percentage of vitamin C released in function of time
Sample VS2M Hydrogel CHITOSAN^{MC}-Vit. C prepared from CHITOSAN^{MC} with swelling degree (α) = 3500%

DETAILED DESCRIPTION OF THE INVENTION

The present detailed description reveals dermatological and dietary compositions comprising one or more active ingredients, photo or thermo sensitive to degradation. These compositions comprising a polyionic hydrogel, generated from a chitosane and xanthane complex, able to incorporate and protect the photo and thermo sensitive active

ingredients, as well as to improve their activity through a controlled release effect.

Two processes for the incorporation of the active ingredients into the chitosane and xanthane hydrogel are also described. The liposoluble active ingredients are incorporated into the hydrogel by a first method, in the course of the formation of the hydrogel. The hydrosoluble active ingredients are incorporated into the hydrogel, by a diffusion method, following the formation of the hydrogel.

The present invention also demonstrates, in a surprising and innovating manner, the possibility of incorporating various vitamins or other ingredients such as amino acids, nucleic acids and oligopeptides into a hydrogel and to then release these substances via different paths such as oral dosages, suppositories, cremes, ointments, gels, solutions, transcutaneous devices ("patch"). Additionally, the present invention reveals the possibility of incorporating and to solubilize in a same hydrogel liposoluble or hydrosoluble vitamins.

Finally a process for the preparation of dietary complement and a dermatological crème incorporating the hydrogel of the present invention.

It is important to recognize that the terms "dermatology" and "dermatological" are used in a broad sense, therefore including all other cosmological and cosmetic applications. In addition, these terms extend themselves to applications on the skin or on the phanera.

The term "dietary complement" also extends itself in a broad sense, therefore including any dietary preparation, the compliment being used in a nutritional or therapeutic role or in a yet simple physical role in dietary preparations, for example, as a texturing agent, as a filling agent or as a viscosity controlling agent.

The compositions of the present invention are primarily destined for humans but may also find application in the veterinary field.

Referring to figure 1, one can note that the hydrogel of the present invention is a chitosane and xanthane based complex, resulting from an ionic reaction between these two polyions. A process for preparing the hydrogel is described in US patent 5,720,206 granted to the same assignee as the present invention. As illustrated in figure 1, a complex is formed between xanthane and chitosane, that is, the establishment of various ionic bonds between chitosane and xanthane molecules, so forming the hydrogel.

Incorporation of liposoluble vitamins in the chitosane-xanthane hydrogel

Vitamin A

Vitamin A (Retinol) is a very light and oxygen sensitive product. The use of this product, for example, in a crème can't be accomplished unless it is stabilized beforehand against the harmful effects of the agents previously cited. The process of the present invention consists in stabilizing vitamin A in a hydrogel composed of xanthane and chitosane, prepared according to the method described in US patent 5,620,706.

Example 1 inclusion of vitamin A during the preparation

A solution (100 ml) of vitamin A (10-20% w/v) in ethanol is first prepared. This solution is then added, under vigorous stirring, to a xanthane solution (500 ml) 0.65% w/v, to finally obtain a final concentration in vitamin A of 1.66 - 3.33% w/v. The solution can be preserved at 3 °C. A pulverization system is then employed in order to add the vitamin A – xanthane solution to a chitosane solution (0.65% w/v). The reaction has to be sustained for 30 min. The so-formed gel has to be filtered and washed with water in order to reach a pH of 6.8. To increase the final stability of the gel, a final washing with a sodium bicarbonate solution (1% w/v) is effected, which brings the gel to a pH of 7.5. The gel

is then frozen and lyophilized. All the operations, including the freezing, are carried out in the absence of oxygen and light.

Example 2 Inclusion of vitamin A by diffusion

It is possible to introduce vitamin A by diffusion, by using a xanthane-chitosane based hydrogel possessing a degree of swelling (α) of at least 2000%. The degree of swelling is defined according to the following equation:

$$\alpha = 100\ \% \times \frac{\text{weight of swelled hydrogel at equilibrium - weight of dry hydrogel}}{\text{weight of dry hydrogel}}$$

Under these conditions the operating times are significantly reduced, in the process preventing the degradation of the molecule. To accomplish this, it suffices to dissolve 0.07 g of vitamin A in 1 ml of ethanol (96%) and to add to this solution 1.5 g of the xanthane – chitosane complex lyophilized with (α) = 2500%. A light stirring allows for the achievement of a homogeneous paste. This is followed by the addition of ethanol (2 ml) and water (200 µl), while maintaining a light stirring. The whole is then placed at 4°C for 24 h shielded from light. The alcohol is then evaporated at 4 °C. The final product possesses a vitamin A concentration of 46 mg / g lyophilized product.

Example 3 Inclusion of vitamin E

For the incorporation of Vitamin E, the process (1) developed for vitamin A is applied. The concentration of vitamin E can reach up to 20%.

Example 4 Inclusion of vitamin K

As far as vitamin K is concerned, the same process (1) as for vitamin A is used. The concentration of vitamin K can reach up to 20%.

Incorporation of water soluble vitamins

For water-soluble bio-active products, it is preferable to use the diffusion process for the incorporation of these products into the lyophilized hydrogel, in order to avoid inevitable losses of products during the reaction between xanthane and chitosane. Under these conditions, it is essential to have a hydrogel with a degree of swelling of at least 2000%.

Example 5 Inclusion of vitamin C

Since vitamin C presents a strong redox potential with regards to chitosane, a novel process of inclusion was developed. This process comprises two (2) steps:

Step 1 The preparation of the xanthane – chitosane complex (CHITOSANE^{MC}), that is the polyionic hydrogel;

Step 2 The incorporation of vitamin C

1. Preparation of the xanthane – chitosane complex

The CHITOSANE^{MC} complex is prepared according to the method described earlier in US patent 5,620,706. The chitosane used in the preparation of this complex, typically has molecular weight ranging from 250,000 to 350,000, and the hydrogel has a degree of swelling (α) of $\geq$ 2000%.

The CHITOSANE^{MC} is ground in order to obtain a fine powder, with particles having a diameter ranging between 250 and 500 µm.

2. Incorporation of vitamin C

2a. Stabilization by amino acids

To 10 ml of water is added vitamin C (1 g), L-cysteine (0.06 g), L-cystine (0.02 g) and L-methionine (0.02 g). Lyophilized CHITOXANE^{MC} (1 g), having a particle size ranging from 250 - 500 µm, is

then added. The mixture cannot contain any excess liquid phase. If necessary, water can be added. The mixture is maintained for 2 hours in order to achieve the hydration equilibrium. All the operations have to be carried out in the absence of light. The mixture is then frozen and lyophilized. The transformation into a final powder like product, yields particles varying in size from 50 to 125 μm.

The so-incorporated hydrated vitamin C presents a stability without any color change of 2 weeks at 45 °C, and of 20 weeks for the dry gel at the same temperature. It is possible to use tartaric acid (0.1%), metaphosphoric acid (0.03%), or citric acid (0.1%) as a stabilizing agent. The weighted percentage is in relation to the CHITOXANE^{MC} complex.

2b. Stabilization by tripeptides

Method 2a is used, by replacing the amino acids by a tripeptide with sulfurized amino acids. To 10 ml of water is added vitamin C (1 g) and glutathione (0.002 g). After stirring for 5 minutes, CHITOXANE^{MC} (1 g) is introduced and the mixture lightly stirred until a homogenous paste is obtained. The mixture is maintained for 2 hours in order to arrive at the hydration equilibrium. The paste is then frozen and lyophilized. All the operations have to be carried out in the absence of light.

Extraction and dosage of vitamin C in CHITOXANE^{MC}

Extraction

Extraction solvent: aqueous solution of metaphosphoric acid (3% w/v) and acetic acid (8% v/v)

Process: In a 50 ml centrifugation tube protected against light, was added from about 20 to 30 mg of lyophilized CHITOXANE^{MC} + Vit. C and 40 ml of the extraction solvent. The whole was stirred for 60 minutes with a magnetic stirrer. The suspension was finally centrifuged (4000 rpm) and the supernatant analyzed.

Quantitative determination of vitamin C

Before proceeding at the quantitative determination of vitamin C, the maximum absorption has to be measured in a UV-VIS spectrophotometer. The standard is prepared by using a vitamin C solution, which served in the manufacture of CHITOXANE^{MC} - Vit. C, in the extraction solvent. The test revealed a maximum absorption at 243 nm for vitamin C of the Aldrich type, purity 98%.

After having evaluated the maximum absorption, the standard absorption curve is determined (absorption in function of concentration of vitamin C). A solution of vitamin C of a concentration of 1.3 mg/ml was prepared in a gauged flask with the extraction solvent. The solution is prepared just prior to the analysis procedure. The absorption / concentration (mg/ml) dependency was measured by successive dilutions.

The concentration of the supernatant obtained in the extraction is determined by photocolorimetry at 243 nm and is calculated from the standard curve.

The concentration of vitamin C in the sample prepared according to process 2a is 49.6%.

Inclusion of CHITOXANE^{MC}-Vit. C in a cream base

CHITOXANE^{MC}-Vit. C (1 g) is hydrated with water, until a cream like paste is obtained. It is then sufficient to weigh the paste and to calculate the obtained concentration in vitamin C. The paste is subsequently incorporated in the cream base while stirring vigorously, in order to obtain a final concentration in vitamin C, preferably about 5 to 25% by weight.

Determination of the stability of vitamin C included in a cream base.

The preparation obtained by inclusion of CHITOXANE^{MC}-Vit. C in a cream base (10 g) is introduced in a light protected tube. This tube is then heated at 45 °C. To determine the degree of degradation of vitamin

C in the cream base, a sample corresponding to about 10-30 mg of vitamin C was drawn. This sample was drawn every day for the first 4 days and then every second day for a total of 30 days. If the samples are not immediately analyzed, they have to be kept refrigerated (-4 °C).

The drawn samples are analyzed as described previously (always using the extraction solvent).

Creams having a concentration ranging from 5 to 25% by weight in vitamin C are prepared from samples VS2L, VS2R and VS2M. By using the described method, a very good stability for vitamin C included in CHITOXANE was found: no coloration of the cream after heating at 45 °C for 30 days while the decomposition of vitamin C was 15%. Under the same conditions, for a cream prepared from free vitamin C, a decomposition of 98% and an orange discoloration could be observed.

Kinetics of release

Referring to Table 1 below, three types of CHITOXANE^{MC}-Vit. C hydrogels were studied. As illustrated by figure 2, the release kinetics of vitamin C are determined by introducing a precise amount of CHITOXANE^{MC}-Vit. C in the reactor. The kinetic release curves were obtained at ambient temperature in the presence of light, by using a vitamin C stabilizer comprised of metaphosphoric acid (3% / vol) and acetic acid (8% / vol).

Table 1

Code for CHITOXANE^{MC}-Vit. C preparations	Degree of swelling (α) of CHITOXANE^{MC} (%)	Concentration of vitamin C in CHITOXANE^{MC}-Vit. C (%)
VS2L	1800	50.3
VS2R	2200	49.6
VS2M	3500	51.0

The solvent (a mixture composed of 3% w/v metaphosphoric acid and 8% w/v acetic acid) penetrates directly into the reactor via a central pipe, where it enters into contact with the CHITOXANE^{MC}-Vitamine C. The vitamin C is gradually released from the complex and dissolves in the solvent. The solvent is drained from the reactor via small apertures, into an exterior pipe. By using this method, a constant circulation of the solvent around the sample to be analyzed, is assured.

It can be observed, by reference to figure 3, that there exists a linear relationship between vitamin C released and time (zero order kinetics), with a clean break after 60 minutes for CHITOXANE^{MC}-Vit. C of type VS2L and with a clean break after 40 minutes for CHITOXANE^{MC}-Vit. C of type VS2R.

It can be observed, by reference to figure 4, that in a first case (CHITOXANE^{MC}-Vit. C of type VS2L), vitamin C diffuses at a constant rate of 0.36 mg/min over a first period, and that the rate decreases by half for the next 100 minutes.

In a second case, as can be observed in figures 5 and 6, the diffusion takes place at a considerably faster rate during the first 40 minutes with a rate of 1.18 mg/min, and is then almost non-existent with a rate of 0.02 mg/min.

Figure 5 illustrates the weight of vitamin C released in function of time for coded sample VS2R Hydrogel CHITOXANE^{MC}-Vit. C, prepared from CHITOXANE^{MC} possessing a degree of swelling (α) = 2000%. Figure 6 illustrates the release rate in function of time of vitamin C, for coded sample VS2R.

Figure 7 illustrates the variation in function of time of the percentage of released vitamin C for a sample VS2M Hydrogel CHITOXANE^{MC}-Vit. C, prepared from CHITOXANE^{MC} possessing a degree of swelling (α) = 3500%. A release in vitamin C of 85% is noted for this sample, during the first 10 minutes of elution.

Inclusion of CHITOXANE^{MC}-Vit. C in a dietary preparation

The CHITOXANE^{MC}-Vit. C hydrogel and other chitosane-xanthane hydrogels containing vitamins, nucleic acids, amino acids and oligopeptides, can also be used in hydrated dietary preparations such as gels, sauces and syrups as well as dehydrated preparations.

The physical characteristics of the selected chitosane-xanthane hydrogel, will determine the structure and the more or less viscous texture of the hydrogel. According to the present invention, the choice of hydrogel will therefore allow for the hydrogel to be adapted to various dietary applications.

In addition, the present invention comprises the formation of pellets from CHITOXANE^{MC}-Vit. C powder with other active ingredients, if required. The formation of pellets from the powder of other lyophilized chitosane-xanthane hydrogels containing various vitamins, amino acids, nucleic acids, oligopeptides or a combination selected from these active ingredients, is also provided for.

It is to be understood however, that various changes and modifications within the spirit and scope of the present application as described, that do not have a direct and material effect upon the way the

invention works will become apparent to those skilled in the art and are to be covered by the following claims.

Claims

1. A thermo and photo stable composition comprising a hydrogel generated from a xanthane and chitosane complex, said hydrogel comprising at least one thermo or photo sensitive substance selected from the group consisting of: vitamins, amino acids, nucleic acids and tri-peptides, said hydrogel being adapted to release said thermo or photo sensitive substances over several hours in a human or animal subject.

2. The composition as defined in claim 1, wherein said thermo or photo sensitive substances are vitamins in a proportion of 5 to 25% of the total weight of said composition.

3. The composition as defined in claim 2, wherein said vitamins are selected from the group consisting of: A, B, C, D, E and K.

4. The composition as defined in claim 3, wherein said vitamin is vitamin A.

5. The composition as defined in claim 3, wherein said vitamin is vitamin C.

6. The composition as defined in claim 1, wherein said thermo and photo sensitive substances are liposoluble.

7. The composition as defined in claim 1, wherein said thermo and photo sensitive substances are hydrosoluble.

8. A process for the preparation of the composition as defined in claim 6, comprising the following steps:

(a) dissolving the liposoluble substances in ethanol;

(b) adding this solution, while stirring, to a xanthane solution;

(c) pulverizing the mixture from step (b) into an aqueous chitosane solution;

(d) recovering the so-formed hydrogel;

(e) incorporating the liposoluble substances into the hydrogel by diffusion of the in ethanol dissolved liposoluble substances (95%/vol) and this at a concentration of 50%/vol.

All the steps being carried out in the absence of oxygen and light.

9. A process for the preparation of the composition as defined in claim 7, comprising the following steps:

(a) pulverizing a xanthane solution into a chitosane solution;

(b) recovering the so-formed hydrogel, the recovery being characterized by a lyophilization of the hydrogel;

(c) introducing the lyophilized hydrogel into an aqueous solution comprising hydrosoluble substances;

(d) incorporating hydrosoluble substances into the hydrogel, by diffusion of hydrosoluble substances during the swelling of the hydrogel, said hydrogel having a degree of swelling (α) of 2000% or more.

10. The preparation process as defined in claim 9 wherein, the stabilization of said hydrosoluble substances is favored by the addition of amino acids into the aqueous solution during step (c).

11. The process as defined in claim 10 wherein, said amino acids are selected from the group consisting of: L-cysteine, L-cystine, L-methionine or mixtures thereof.

12. The preparation process as defined in claim 9 wherein, the stabilization of said hydrosoluble substances is favored by the addition of tri-peptides during step (c).

13. The use of the composition of any one of claims 1 to 7 in the manufacture of a dermatological product.

14. The use as defined in claim 13 wherein, said dermatological product is a cream.

15. The use as defined in claim 14 wherein, said thermo and photo sensitive substance is vitamin A.

16. The use of the composition of any one of claims 1 to 7 in the manufacture of a dietary supplement.

ABSTRACT OF THE DISCLOSURE

In general, the invention relates to a thermo and photo stable composition comprising a hydrogel generated from xanthane and chitosane, the hydrogel comprising at least one thermo or photo sensitive substance selected from the group consisting of: vitamins, amino acids, nucleic acids and oligopeptides, the hydrogel being adapted to release said thermo or photo sensitive substances in an animal or human subject. The invention also discloses a process for the manufacture of these hydrogels. Additionally, the invention discloses a method of use for these hydrogels in dermatology or as a dietary food compliment.

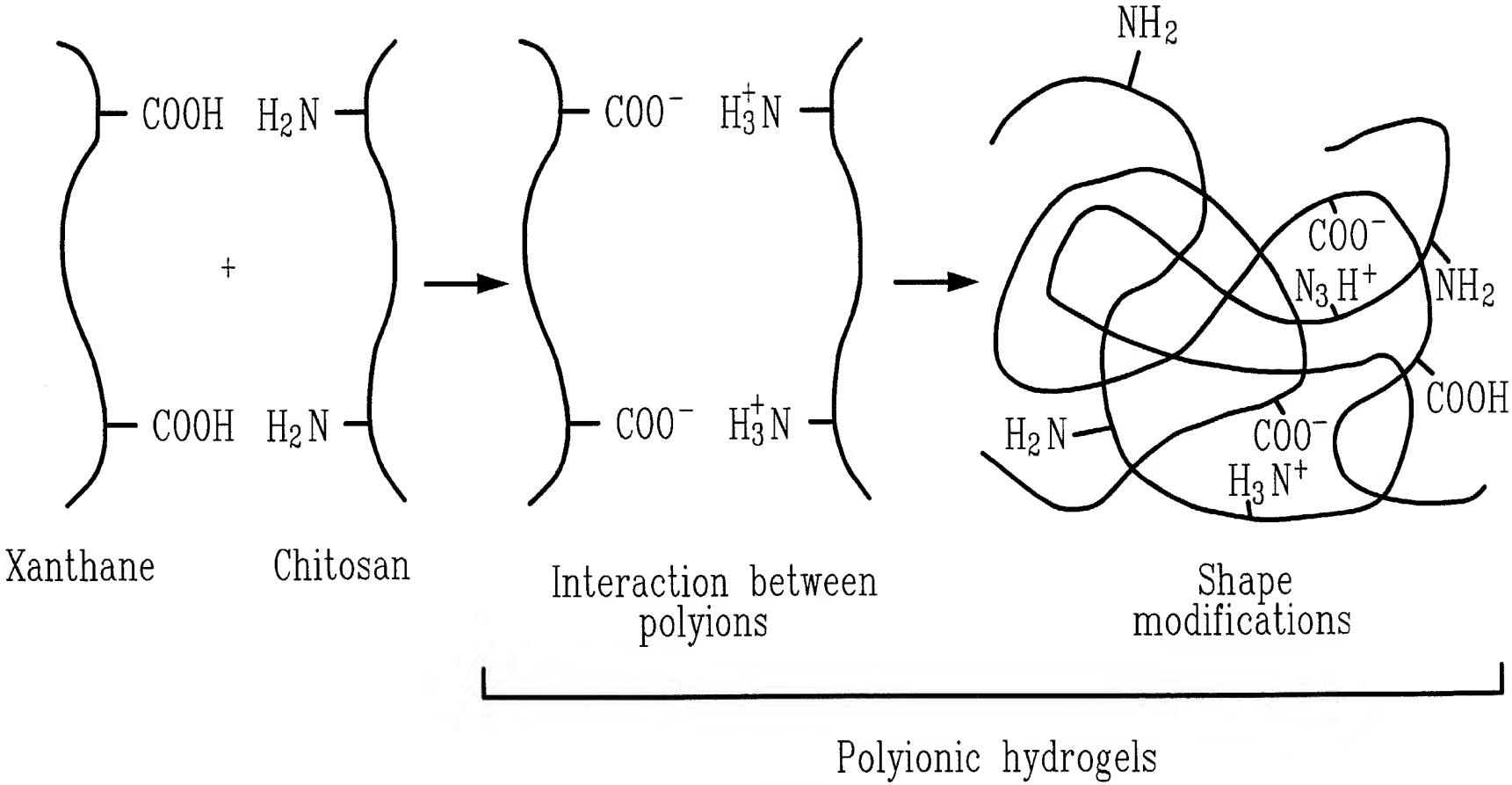

Fig - 1

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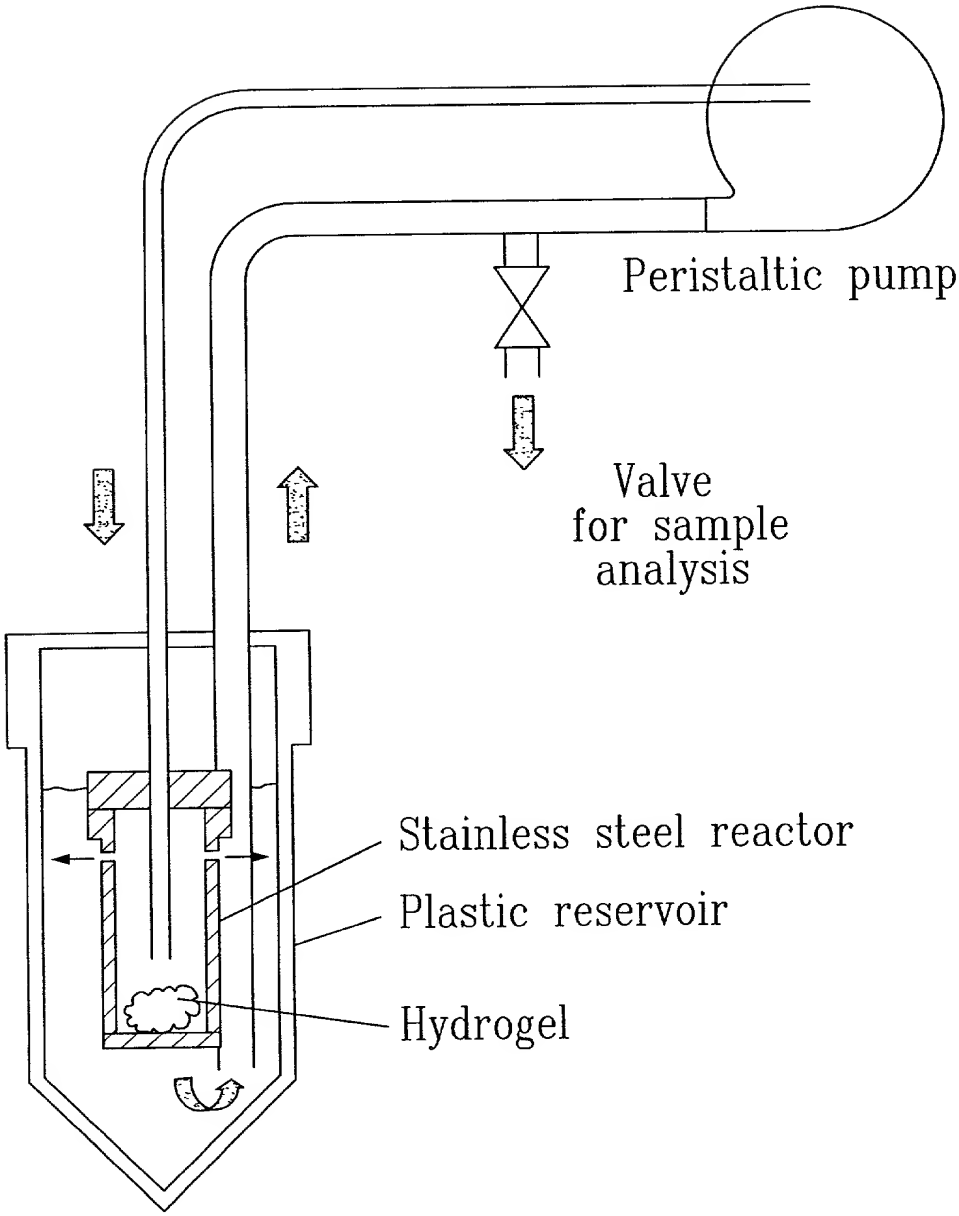

FIG. 2

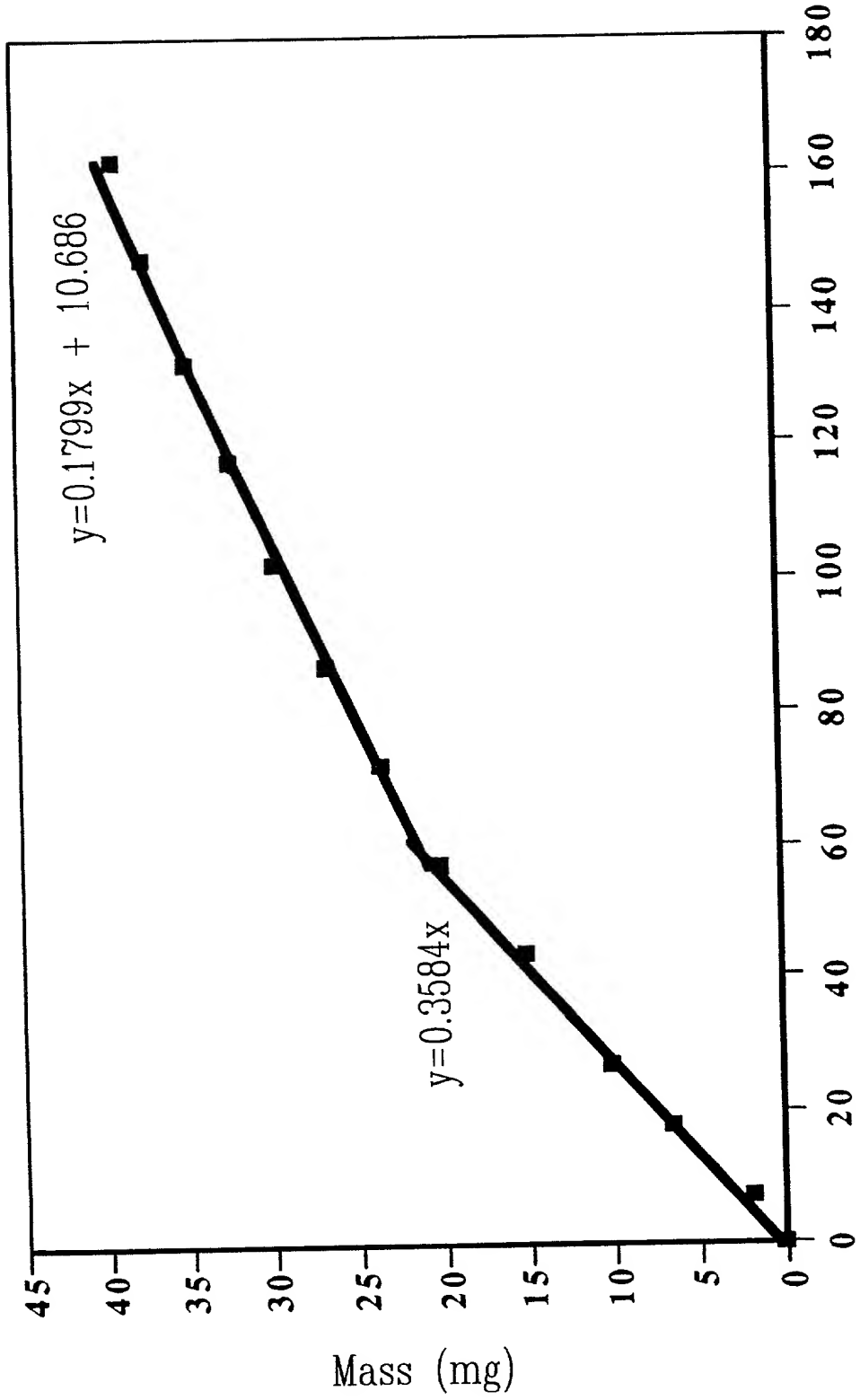

Fig - 3

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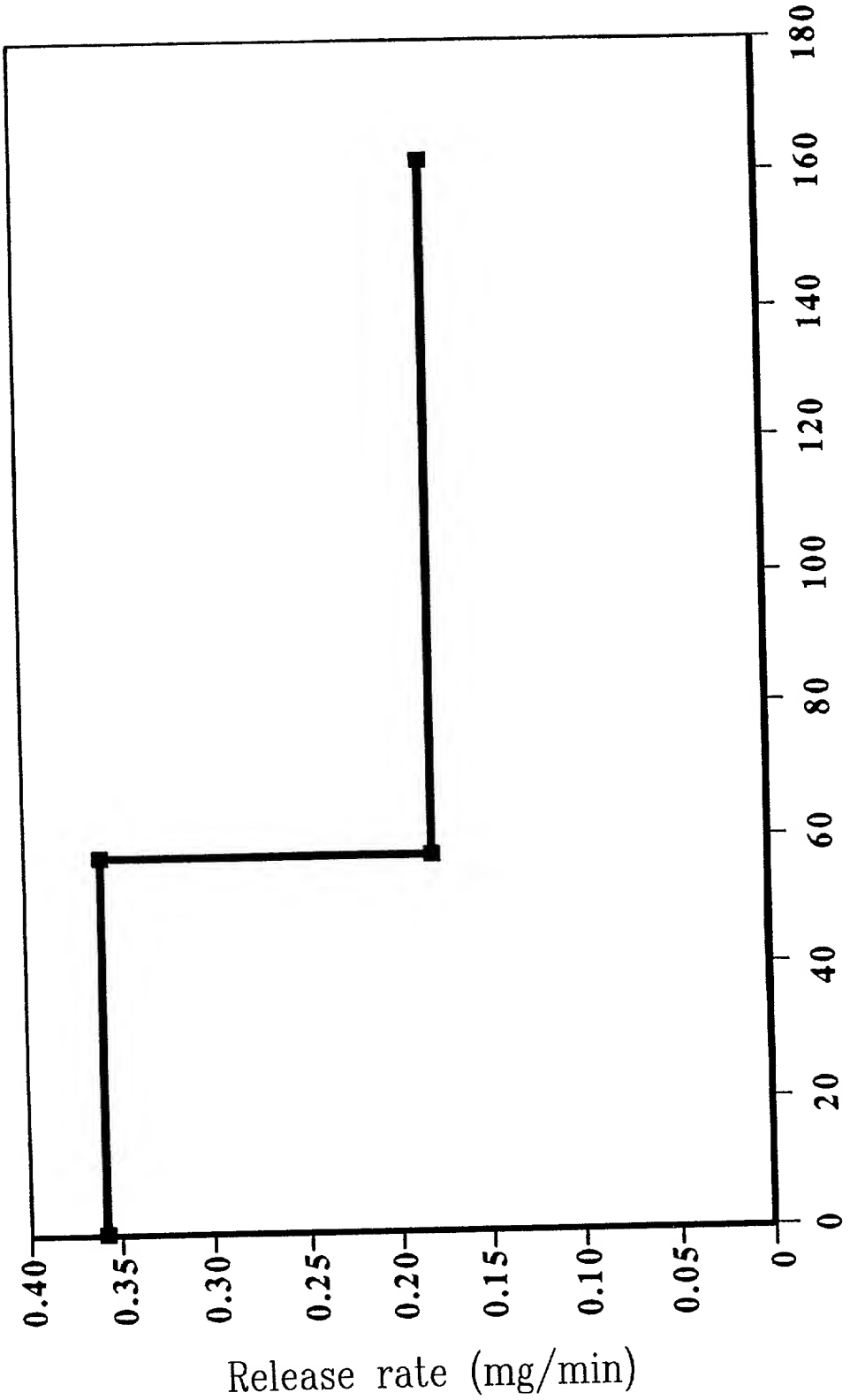

Fig-4

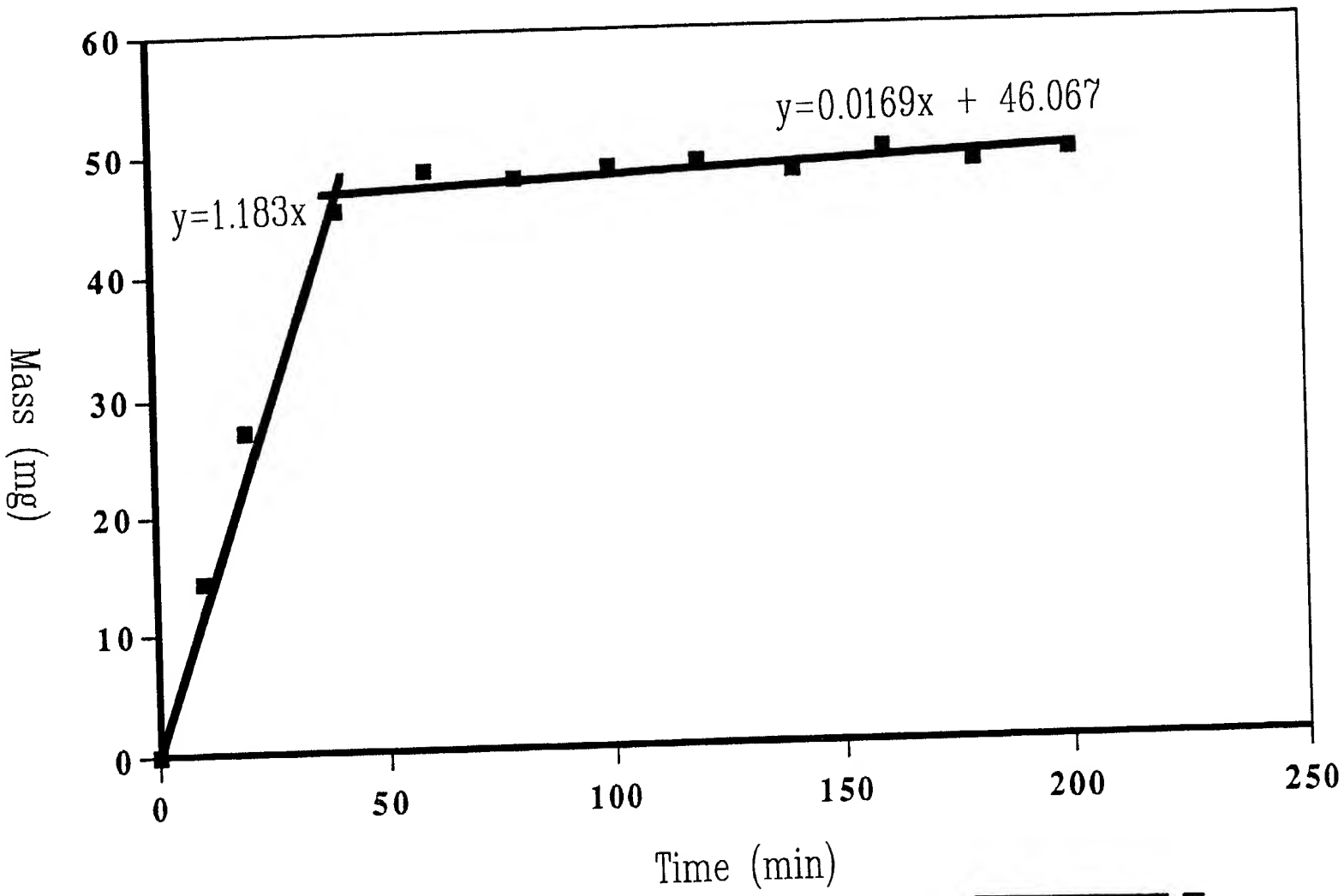

Fig. 5

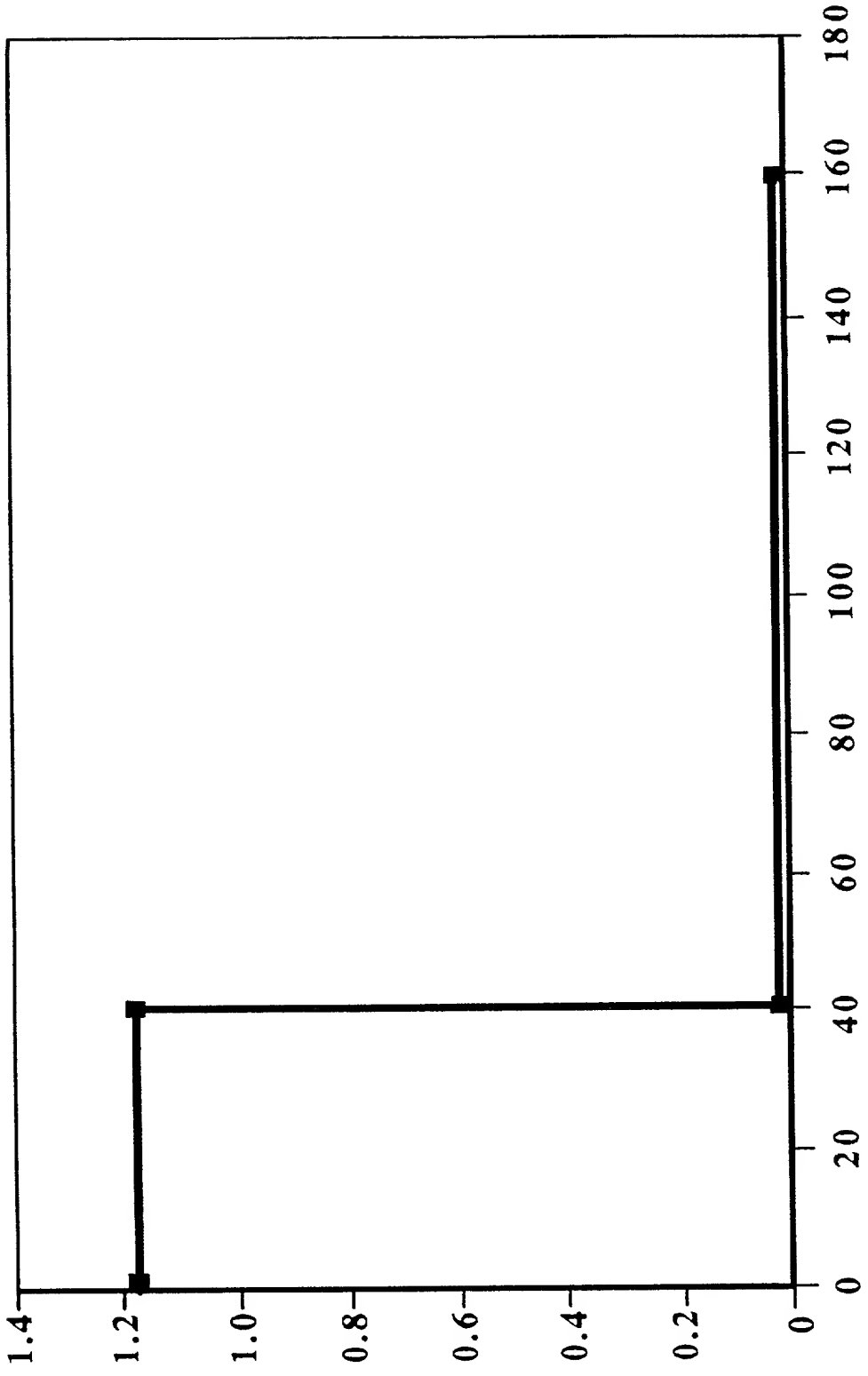

FIG - 6

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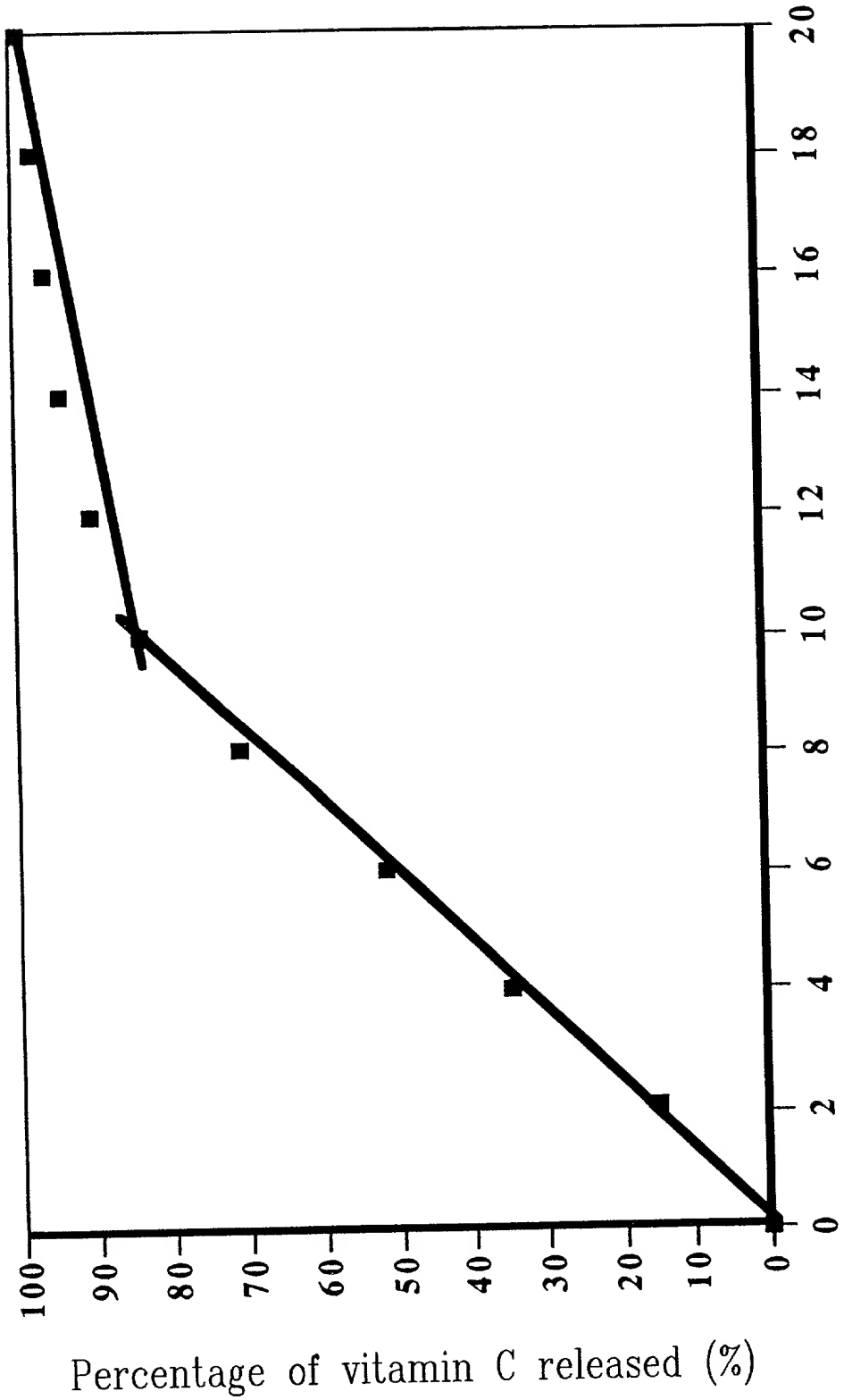

Fig. 7

Docket No.
3203.17330-PCT US

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Polyionic Hydrogels Containing Xanthane and Chitosan

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **16 July 1999** as United States Application No. or PCT International Application Number **09/743,930 (PCT/CA99/00651)**

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
2,243,619	**Canada**	**17 July 1998**	☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/CA99/00651	**16 July 1999**	**Pending**
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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